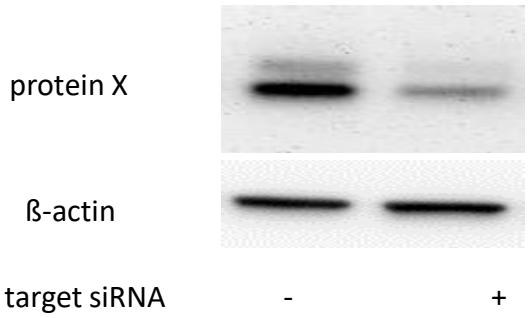
C2C12: transfection of siRNA



VB-01LB-00, VB-01LB-01

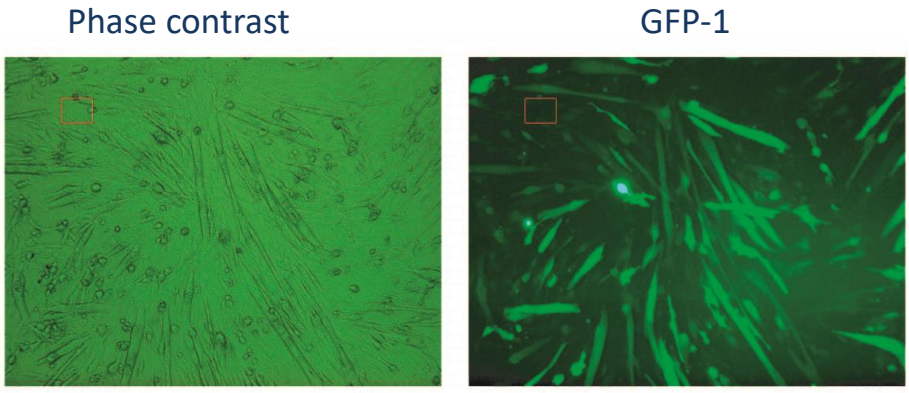
Fig. 1: Knockdown of gene X expression in C2C12 myoblasts transfected with Viromer® BLUE

- cells cultivated in DMEM, 20% FCS and Gentamycin
- 100nM siRNA (standard protocol)

Data from Prof. J. Hall & J. Zagalak, Institute for Pharmaceutical Science, ETH Zurich (Switzerland)

Important note:
High transfection efficiency only achievable before cell differentiation.

C2C12: transfection of plasmid DNA



VR-01LB-00, VR-01LB-01

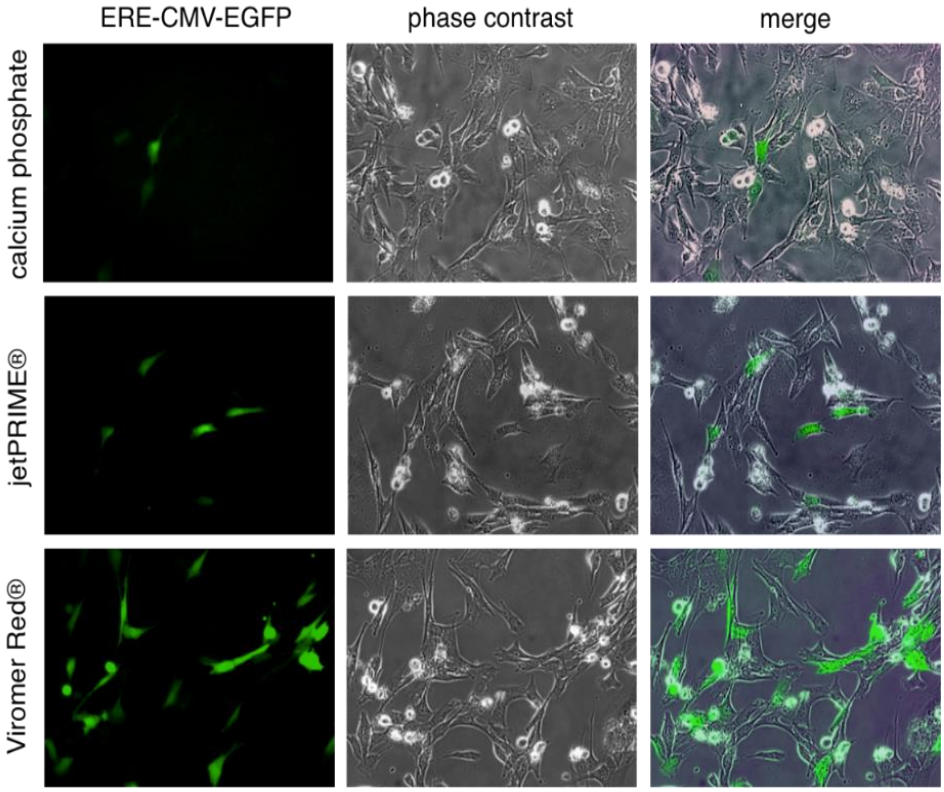
Fig. 1: Expression of GFP in C2C12 cells transfected with Viromer® RED

- 6-well plate format, standard protocol:
- C2C12 seeded in antibiotic-free medium
- after overnight, 80% confluence achieved
- pDNA:Viromer complexes added on cells with antibiotic-free medium
- after 24h-48h, wait for the cells to be at 90-100% confluence
- shift to cell fusion medium (dMEM+2%HS+PS)
- change to fresh fusion medium everyday (until day 4 → picture/assay)

"We have tried the Viromer on undifferentiated C2C12. The myotube remains being transfected and it works great"

Data from Dr. CL Tse, Health Science Center, Dept. of Physiology University of Oklahoma (USA)

C2C12: transfection of plasmid DNA



VR-01LB-00, VR-01LB-01

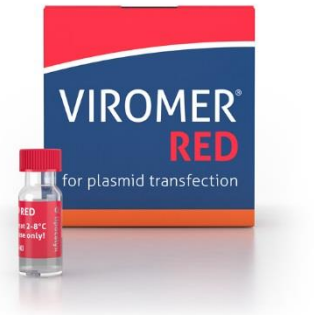
Fig. 3 (Fig. 27 in Master thesis, L. Pinto, 2015): Pictures of EGFP fluorescence were taken 24h after transfection of C2C12 myoblasts with the plasmid ERE-CMV-EGFP using 3 methods. To help evaluating the percentage of cells that express EGFP, phase contrast pictures of the cells were also taken and superimposed to the fluorescence ones.

"The same experiment was repeated several times. Overall, with the ERE-CMV-EGFP plasmid, around 40-50% of the myoblasts showed green fluorescence with Viromer Red®. The efficacy of jetPRIME® was variable and usually quite low. Only in one experiment jetPRIME® was almost as efficacious as Viromer Red®. Based on these findings, we selected Viromer Red® as the transfection reagent for further experiments with C2C12 myoblasts..."

- 12-well plate format
- 1 µg DNA/well (2 µg for JetPRIME® and calcium phosphate transfections)
- No toxicity observed
- **40-50% efficiency**

Master thesis of Laeticia Pinto – June 2015, EPGL (Switzerland)

C2C12: transfection of plasmid DNA



VR-01LB-00, VR-01LB-01

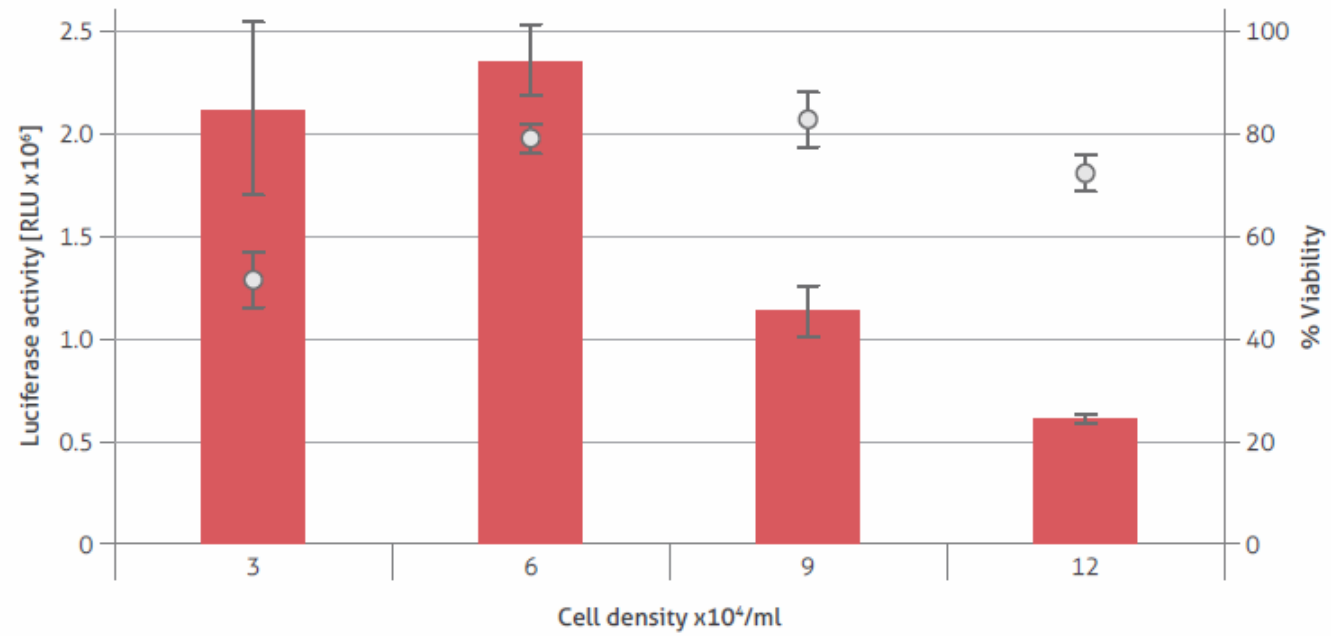


Fig. 4: Comparison of luciferase activity and cell viability 24h post-transfection of pDNA (6kb) with Viromer® RED at 4 different seeding densities

– Basic standard protocol in 96-well plate (n=6).
>> The observed optimal density (6x10^4 cells/ml) is under the recommended range (8-15x 6x10^4 cells/ml), as C2C12 are large cells, form monolayers, and have a rapid growth (doubling time ≈12h)

Internal data Lipocalyx GmbH

C2C12: transfection of pDNA and mRNA

VR-BUNDLE-01

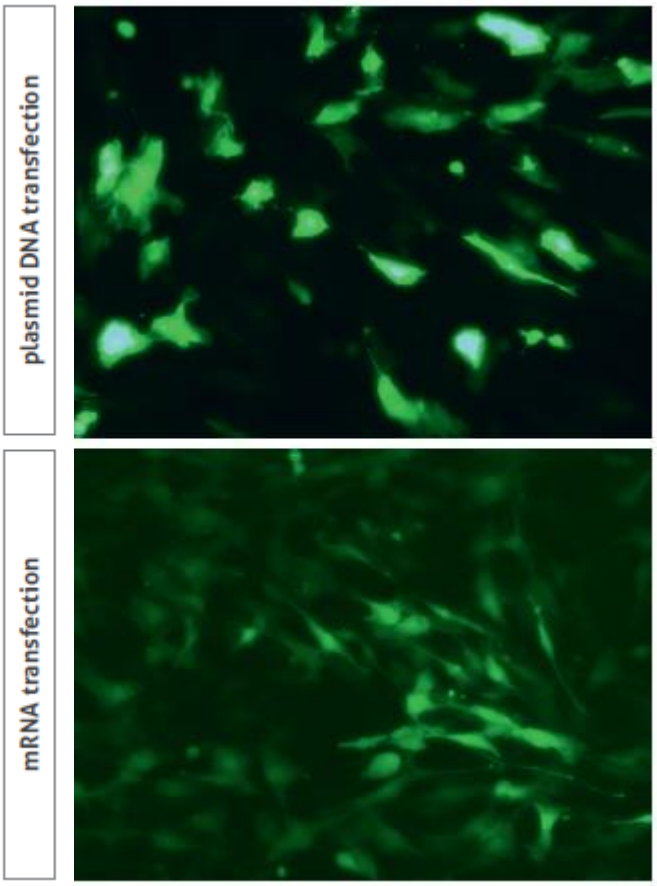


Fig. 5: Comparative expression of GFP after transfection of C2C12 cells with plasmid or mRNA using the Viromer® RED Start Positive® Controls

- transfection with pCMV-GFP plasmid and GFP-mRNA
- monitored by fluorescence microscopy
- Faster, homogeneous and stronger expression with mRNA than with plasmid DNA (instant availability of the transcript is assumed)

Data from H. Cynis, Fraunhofer Institute for Cell Therapy and Immunology, Halle (Germany)